

## Comparison between uterine artery and sub-endometrial blood flow before and after Nitric oxide donors in women with recurrent miscarriage

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**Abstract: Background:** Recurrent miscarriage represents a major concern for reproductive medicine. Nitric oxide plays a major role in improvement of uterine perfusion during the luteal phase and early pregnancy. This study was done to compare uterine artery and sub-endometrial blood flow indices during the luteal phase between patients with recurrent miscarriage and fertile women and also to evaluate the effects of nitric oxide donors on blood flow indices in the patient group. **Methods:** The study included a control group of 40 women with good obstetric history (delivered at least one child), do not have abnormal findings in routine examinations for female factor and are not primigravidae and a patient group of 40 nulliparous women with history of two or more unexplained recurrent miscarriage. Transvaginal three-dimensional pulsed color Doppler ultrasound was done on days 21 of the cycle to measure uterine artery resistance and pulsation indices. The effect of nitric oxide donors was evaluated in the study and control group by placing 20 mg Isosorbide Mononitrate vaginally. Pulsatility index (PI) and Resistance Index (RI) of uterine artery were measured after two hours. The Student t-test and the paired t-test were used for analysis of results and a p-value of  $\leq 0.05$  was considered significant. **Results:** Patients with unexplained recurrent miscarriage had significantly higher uterine artery resistance and pulsation indices ( $p < 0.01$ ). Isosorbide mononitrate significantly decreased uterine artery blood flow indices ( $p < 0.001$ ). **Conclusion:** Uterine artery blood flow decreased during the luteal phase in patients with unexplained recurrent miscarriage. Nitric oxide donors increased blood flow and may be of a therapeutic value.

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**Keywords:** Recurrent Miscarriage, Uterine artery blood flow, Nitric oxide donors.

### 1. Introduction

Recurrent early miscarriage is defined as three or more successive pregnancy losses before 20 weeks of gestation and affects about 0.5-3% of couples (1). Recently, it was defined as two or more (2) and this increases the incidence up to 5%. Treatment of RA is a real challenge (4) and in most cases is unsuccessful as identifiable causes can be found only in 30-50% of women (5) and the rest remain unexplained. Scientists showed that during the luteal phase of normal menstrual cycle, uterine artery impedance blood flow decreases and there is an increase in uterine and sub-endometrial blood flow which reaches its maximum level during the period of implantation (6). Other studies (7) showed that uterine artery perfusion regulates uterine receptivity, influences the success of implantation, maintains early pregnancy and that impaired uterine perfusion plays a central role in the pathogenesis of unexplained recurrent miscarriage (8).

Past studies (9, 10) provide evidences that nitric oxide (NO) generated in vivo from the essential amino acid L-arginine by the vascular endothelium plays a major role in vascular smooth muscle relaxation. Subsequently, it results in a decrease in vascular resistance which leads to an increase in uterine artery

blood flow observable in early pregnancy. Impaired L-arginine-nitric oxide pathway has been suggested to be a subtle cause of unexplained recurrent miscarriage and treatment with nitric oxide donors is reported as a significant success in some studies (11-13).

Based on these observations, an attempt was made to study uterine arteries and sub-endometrial blood flow during the luteal phase in normal fertile women and in patients with unexplained recurrent miscarriage. Moreover, in this study, an investigation was carried out to find the effects of vaginal administration of Isosorbide mononitrate (IMN) as a source of nitric oxide and a perfusion enhancer on uterine arteries and subendometrial blood flow indices in these patients.

### 2. Methods

The study was done during the period from January to November 2017 at El Hussein Hospital of Al-Azhar University in Cairo-Egypt after approval of the ethical committee and informed consent was taken from every participant in two groups. The control group consists of 40 women with good obstetric history (delivered at least one child), do not have abnormal findings in routine examinations for female

factor and are not primigravidae and a patient group of 40 nulliparous women with history of two or more unexplained recurrent miscarriage which entails presence of a normal uterine cavity by hystero-graphy, no luteal phase defect (progesterone level >10 ng/ml), normal thyroid function tests (TSH, T3 and T4), normal levels of lupus anticoagulant measured by the activated partial thromboplastin time (32-43 seconds), normal levels of anticardiolipin IgG (<20 GPL) and IgM (<15 MPL) measured by ELISA and normal karyotyping (done for 10 cases with more than three successive abortions). Women using hormonal contraception or intrauterine device or treated with vasodilators were excluded. Vaginal ultrasound (Voluson®, PRO 720, and GE Medical Systems) was done on cycle days 21 of both groups using 7.5 M. Hz probe; Transvaginal three-dimensional pulsed color Doppler ultrasound identified the uterine artery and three similar consecutive waveforms were displayed and the resistance index (RI) and the pulsation index (PI) were measured.

Isosorbidedimonitrite (IMN) 20 mg tablet (Effox, Mina Pharma Co, Egypt; under license of

Schwartz Pharma, Germany) was administered vaginally in the study group and blood flow indices were measured again after two hours.

#### Statistical design:

Data were analyzed using SPSS software version 20. The mean, standard deviation and the Student t test were used to compare blood flow indices in the control and abortion groups and the paired t-test was used for comparison of blood flow before and after administration of IMN in the abortion group. The  $p \leq 0.05$  was considered statistically significant.

### 3. Results

There was no significant difference between both groups regarding the mean age, the mean blood pressure and the mean body mass index ( $p > 0.05$ ) as shown in table 1.

However, there was a significantly higher uterine artery RI and PI ( $P < 0.01$ ) as shown in table 2.

After administration of IMN in the patient group, there was a significant decrease of uterine artery RI and PI as shown in table 3.

**Table (1)**

Parameter	Patient group (n = 40)	Control group (n = 40)	t test	P value
	Mean ( $\pm$ SD)	Mean ( $\pm$ SD)		
Maternal age (yrs)	35.2 ( $\pm$ 1.5)	34.4 ( $\pm$ 1.6)	1.73	0.087 (NS)
Mean arterial pressure (mmHg)	85.78 ( $\pm$ 5.9)	84.9 ( $\pm$ 6.7)	0.6	0.55 (NS)
BMI (Kg/m <sup>2</sup> )	24.31 ( $\pm$ 2.3)	23.8 ( $\pm$ 1.9)	0.9	0.36 (NS)

**Table (2)**

Parameter	Patient group (n = 40)	Control group (n = 40)	t test	P value
	Mean ( $\pm$ SD)	Mean ( $\pm$ SD)		
R.I.	0.84 ( $\pm$ 0.03)	0.79 ( $\pm$ 0.02)	8.771	<0.01 (S)
P.I.	2.83 ( $\pm$ 0.05)	2.18 ( $\pm$ 0.10)	36.770	<0.001(S)

**Table (3)**

Parameter	Before (n = 40)	After (n = 40)	t test	P value
	Mean ( $\pm$ SD)	Mean ( $\pm$ SD)		
R.I.	0.84 ( $\pm$ 0.03)	0.76 ( $\pm$ 0.03)	11.926	<0.001(S)
P.I.	2.83 ( $\pm$ 0.04)	2.15 ( $\pm$ 0.2)	21.086	<0.001(S)

### 4. Discussion

Decreased uterine and sub-endometrial artery blood flow is considered a causative factor in recurrent abortion (14). In the present study, the RI and PI of the uterine arteries were significantly higher during the luteal phase in patients with unexplained recurrent miscarriage as compared to normal fertile women ( $p < 0.01$ ). This result agreed with previous studies (15)

which referred to the elevated uterine artery RI in women with unexplained recurrent miscarriage in the mid luteal phase of a non pregnant cycle. In another study (16-13), women with unexplained recurrent miscarriage had significantly higher uterine artery PI in the second half of the menstrual cycle than those in a control group. Vaquero et al. (17) reported significant decrease of sub-endometrial blood flow

indices with an increase in resistance to uterine artery blood flow in patients with unexplained recurrent miscarriage. El-mashad et al. (18) evaluated uterine PI and plasma adrenomedullin (AM) levels in women with unexplained recurrent pregnancy loss (RPL) compared to controls and found that uterine artery PI and AM levels were significantly higher in RPL group. However, Yildiz et al. (19) reported no statistically significant difference regarding uterine artery PI and RI values measured between the 18th and 23rd days of the menstrual cycle in patients with recurrent pregnancy loss and the control group. In the present study, IMN applied vaginally during the luteal phase in patients with history of unexplained recurrent miscarriage caused significant decrease in uterine artery RI and PI. In a study by El Far et al., (11) intravaginal sildenafil citrate-a selective phosphodiesterase-5 (PDE-5) inhibitor that augments the action of nitric oxide by inhibiting cAMP degradation by the enzyme (PDE-5) successfully treated four patients with unexplained recurrent spontaneous miscarriage. Other authors (20) investigated the effect of sildenafil on uterine volumetric blood flow (UVF) and vascular impedance in nonpregnant, nulliparous women in the luteal phase and demonstrated a significant increase in UVF in response to sildenafil. In another study, Amin et al. (12) evaluated the effect of treatment with N-acetyl cysteine as a NO donor plus folic acid in patients with unexplained recurrent pregnancy loss and found a significant increase in the take-home baby rate as compared to treatment with folic acid alone. Amite et al. (26) studied the effect of NO donors on uterine blood flow velocity waveforms during the first trimester of pregnancy by administration of 5 mg IMN sublingual and found a fall in the RI of the uterine artery and concluded that NO donors may be of therapeutic value in cases where uterine circulation is impaired. The results of the present study supported Amite et al.'s conclusion. Nitric oxide has important physiological functions and its use in the luteal phase and during pregnancy is promising for treatment of URA. Nitric oxide increases uterine and sub-endometrial blood flow, increases utero-placental circulation (21), causes peripheral vasodilatation and lowers systemic blood pressure (22). Also, it has anti-thrombotic and anti-inflammatory effects by inhibiting platelet aggregation and adhesions of neutrophils to the vessel wall (23). Moreover, it regulates apoptosis (24) and causes relaxation of the myometrium (25).

### Conclusion

Patients with unexplained recurrent miscarriage had a decrease in uterine arteries and sub-endometrial blood flow during the luteal phase of the menstrual cycle. Nitric oxide donors act as perfusion enhancer

drugs that increase blood flow and may be of value in improving endometrial receptivity and pregnancy outcome.

### Conflict of Interest

The authors declare no conflict of interest.

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